Vol. LXXXI, No. 2 October, 1941

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE RESPONSES OF CATFISH MELANOPHORES TO ERGOTAMINE

G. H. PARKER

(*From the Biological Laboratories, Harvard University*)

Some eight years ago Bacq (1933), on the basis of experimental evidence, reached the conclusion that ergotamine contracts denervated catfish melanophores and expands normally innervated ones. It is now known that the so-called contraction of catfish melanophores is the result of a neurohumor from the concentrating nerve-fibers, very likely adrenaline, and that their expansion is due to two agents, intermedine from the pituitary gland and a neurohumor, probably acetylcholine, from the dispersing nerve-fibers (Chang, Hsieh, and Lu, 1939; Parker, 1940). In consequence of these new discoveries it seemed desirable to repeat Bacq's experiments with the view of bringing his rather remarkable results into relation with this newly acquired information.

The ergotamine tartrate used by Bacq is fortunately still to be had in the American market. It is dispensed in 1 cc. glass ampules under the name of gynergen (Sandoz Chemical Works) and in this form it is extremely convenient for experiments on fishes. Three sets of catfishes (*Ameiurus nebulosus*) were prepared for these tests,—pale, intermediate, and dark. The pale fishes, three in number in the initial set, were kept in white-walled, illuminated vessels. Two caudal bands were cut in each fish. By the end of six days these fishes were very pale and their caudal bands were almost fully blanched. The axis and tip of each band, however, were noticeably dark as observed and figured by Bacq (1933). The three fishes of intermediate tint were kept in a gray, illuminated vessel. Their caudal bands after six days were very slightly darker than the rest of their darkish tails. The three fishes of the dark set were rendered very dark, coal-black, by complete blinding. It is well known that catfishes assume this intense shade on double enucleation. Notwithstanding the great depth of tint thus produced, the caudal bands in these fishes were a shade darker than the rest of their very dark tails.

Six days after the cutting of the caudal bands in the pale fishes these bands were recut a little distal to the original incision. Since the part of the band distal to the new cut did not change in tint as a result of the recutting, it was concluded that so far as color changes were concerned the nerves of such bands had degenerated. As the three fishes in any given set, pale, intermediate, or dark, were very similar in color, one in each set was reserved as a control and the other two were subjected to tests. Two injections each of 0.25 cc. of gynergen separated by an interval of about a quarter of an hour yielded the best results. These injections were at times supplemented by a third. Two injections of 0.5 cc. of gynergen with an interval of fifteen minutes between them gave more vigorous responses than the weaker injections, but they were usually followed some hours later by the death of the fish. I was unable to obtain unquestionable responses with only a single injection of 0.25 cc. of gynergen as reported by Bacq. The catfishes used by me weighed each about 50 grams. Bacq makes no statement as to the weight of his specimens. Possibly he had smaller individuals than I had and therefore obtained satisfactory responses with less ergotamine. In my procedure any given catfish must have received into its body from the two injections ordinarily given a total amount of 0.25 mg. of ergotamine tartrate judged from the formula published by the Sandoz Chemical Works for their preparation of gynergen.

Bacq's tests, which were carried out only on pale catfishes, consisted in injecting into such a fish with a blanched caudal band 0.25 cc. of gynergen whereupon the fish as a whole became dark, but the band remained pale or even took on a somewhat lighter tint. My repetition of such a test gave almost identical results. When a pale fish with two blanched caudal bands was injected with the usual two doses of gynergen, 0.25 cc. each, with an intervening quarter of an hour, the fish began to darken noticeably in about half an hour after the first injection and in an hour to an hour and a half it had reached a full intermediate tint, its maximum color change under the circumstances. As the tail darkened the bands appeared to become paler as noted by Bacq, but whether this was an actual blanching or a contrast phenomenon could not be settled except by close scrutiny. When the bands on the tail of an injected fish were closely compared with those on the uninjected pale control, the two sets of bands were found to be in very close agreement. This was particularly well seen under a low power of the microscope. In both sets of bands the pigment masses in the macromelanophores were rounded bodies with short, blunt protuberances on their sides marking the roots of the pigmented processes of the dispersed stage. The pigment masses in the injected fishes appeared to be in no sense less dispersed than those

in the control fish, and yet when the bands were inspected by the unaided eye those in the dark fishes appeared to be paler than those in the pale control. In my opinion this apparent difference is purely an illusion due to contrast. The dark surroundings of the pale bands in the injected fishes made these bands appear paler than the pale bands in the pale control. I therefore conclude that, contrary to Bacq's view, ergotamine has no effect on denervated melanophores with concentrated pigment. This agent, however, does induce pigment dispersion in innervated color cells, as stated by Bacq.

In one other respect my observations do not agree with those of Bacq. In the majority of caudal bands that have been blanched in pale fishes for some six days the axes and tips of these bands, as already stated, are slightly dark. This feature was described and figured by Bacq, who noted further that when catfishes showing these peculiarities were injected with ergotamine the pale bands not only became paler but their dark axes and tips also blanched. In my experience such was not the case. After full doses of ergotamine had been allowed to act on the two pale catfishes tested by me, the dark axes and tips in their caudal bands were as visible after the injection as they had been before it or as they were in the control.

In making these several comparisons the individual catfishes in the course of inspection were necessarily much handled. As is well known, this treatment induces such fishes to darken temporarily and it might be supposed that this darkening could in some way have influenced the results just described. But both the control fish and the two injected individuals were handled to about the same degree and therefore should have shown the same amount of change as a result of this treatment. Moreover, it has been demonstrated in a recent paper (Parker, 1940) that the darkening already alluded to is a response mediated by the dispersing nerves. Consequently it ought to play no part in the activities of a denervated area such as a caudal band. There is therefore no reason to suppose that the ordinary darkening of catfishes from handling could have had any influence on the results herein recorded.

The tests on the three pale catfishes just described were repeated on two other sets of pale individuals, one of two fishes and the other of three. In both these sets the pale bands of the injected fishes showed no more change in tint than did those of the first set and their bodies in general darkened to intermediate. This agreement in three sets of results justifies the conclusion that, as Bacq maintained, ergotamine excites innervated melanophores in catfishes to disperse their pigment to a point where the fish attains an intermediate tint. It shows further that, contrary to Bacq's opinion, this agent does not induce a concentration of

pigment in denervated melanophores whereby caudal bands in pale fishes would become still paler. Ergotamine apparently has no influence whatever on denervated melanophores with concentrated pigment. It does induce pigment dispersion in innervated melanophores.

When catfishes with caudal bands cut in their tails are kept for some six days in a gray, illuminated vessel they take on, as already stated, an intermediate tint in which the bands are as a rule slightly darker than the rest of the fish. If these fishes now receive the usual injections of ergotamine, two doses of gynergen, 0.25 cc. each, separated by a quarter of an hour, they will either show no noticeable change in tint at all or darken very slightly. This rule held for all three sets of catfishes tested, including a total of nine individuals. In no instance was there any evidence of the blanching of the denervated bands, but, contrary to what might have been expected from Bacq's statements, these bands remained usually a little darker than the rest of the tail. Bacq apparently never tested fishes of intermediate tint with ergotamine. Had he done so, he surely would have observed that when the ergotamine excited any change at all it was a very mild darkening in the region of the innervated color cells and not only no blanching but no change of color whatever in that of the denervated cells.

What has been stated for catfishes of an intermediate tint may be affirmed in general for those that are fully dark. As noted previously, the caudal bands in such fishes are as a rule very slightly darker than the rest of the tails in these individuals. After the usual injections of ergotamine these color conditions either showed no change or the fish as a whole darkened very slightly. In two instances this general darkening was sufficient to make the tail slightly darker than the bands. As comparisons with the control fish showed, this was a real darkening of the tail and not a slight blanching of the band. Hence we are justified in concluding, as in the instance of the intermediate fishes, that ergotamine induces pigment dispersion in innervated melanophores but has no effect on denervated ones.

From these several tests on pale, intermediate, and dark catfishes it seems fair to conclude that ergotamine acts only on innervated melanophores by inducing them to disperse their pigment and has no effect whatever on denervated melanophores. The assumed blanching of these color cells by this agent, as described by Bacq, is purely illusory. So far as the end result is concerned, ergotamine is like intermedine or acetylcholine in that it causes catfishes to darken. But it does not act in the same way as these two neurohumors do. They act directly on the melanophores (Parker, 1941), for they will induce denervated caudal bands to darken. Ergotamine acts on melanophores indirectly, that is through

nerves, in that it excites at some central nervous station the dispersing nervous elements which in turn excite the appropriate nervous terminals to discharge acetylcholine. This neurohumor causes the melanophores to disperse their pigment whereby the fish darkens. Ergotamine is a good example of an indirect melanophoric agent as contrast with direct ones such as intermedine, acetylcholine, and adrenaline. These will activate denervated melanophores in caudal band. Ergotamine is incapable of this activity.

Summary

1. Ergotamine acts on only innervated melanophores by inducing them to disperse their pigment. It is without effect on denervated melanophores either with dispersed or with concentrated pigment.

2. It acts on innervated melanophores only indirectly, that is, through their nerves. These are excited by ergotamine centrally to produce at their melanophore terminals acetylcholine which causes the color cells to disperse their pigment.

3. Ergotamine is a good example of an *indirect* excitant of melanophores as contrasted with *direct* excitants such as intermedine, acetylcholine, and adrenaline, all of which act directly on these color cells.

REFERENCES

Bacq, Z. M., 1933. The action of ergotamine on the chromatophores of the catfish (Ameiurus nebulosus). *Biol. Bull.*, **65**: 387–388.

Chang, H. C., W. Hsieh, and Y. M. Lu, 1939. Light-pituitary reflex and the adrenergic-cholinergic sympathetic nerve in a teleost. *Proc. Soc. Exper. Biol. Med.*, **40**: 455–456.

Parker, G. H., 1940. On the neurohumors of the color changes in catfishes and on fats and oils as protective agents for such substances. *Proc. Am. Philos. Soc.*, **83**: 379–409.

Parker, G. H., 1941. The method of activation of melanophores and the limitations of melanophore responses in the catfish Ameiurus. *Proc. Am. Philos. Soc.* (In press.)